SUSCEPTIBILITY OF THE LONE STAR TICK (*AMBLYOMMA AMERICANUM*) TO INFECTION WITH *THEILERIA CERVI* UNDER NATURAL AND EXPERIMENTAL CONDITIONS

By

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CHAPTER I

INTRODUCTION

Theileria cervi is an intraerythrocytic protozoan parasite of white-tailed deer (*Odocoileus virginianus*) found throughout the southeastern United States including eastern Texas and Oklahoma. The first documented case of *Theileria* sp. in North America occurred in a splenectomized calf during an anaplasmosis study (Splitter, 1950). In 1961, a splenectomized white-tailed deer from Missouri developed a blood piroplasm which was also identified as a *Theileria* sp. (Kreier et al., 1962). The piroplasm was successfully transmitted to another white-tailed deer by intravenous injection of infected whole blood. Attempts to transmit the deer piroplasm to a splenectomized calf and a sheep were unsuccessful. Schaffler (1962) designated this organism as *T. cervi* based on a morphologic comparison of the erythrocytic piroplasms observed in the white-tailed deer and those described in fallow deer (*Dama dama*) by Bettencourt, et al. (1907). Shaffler described the piroplasm shapes as commas, safety pins, bipolar and signet rings.

Robinson et al. (1967) indicated that losses in free-ranging white-tailed deer in Texas attributable to *T.*

cervi coincided with periods of nutritional deprivation or concomitant disease. He concluded that inadequate nutrition rendered the deer susceptible to disease with T. cervi. Field studies conducted in Texas also indicated that areas with a high prevalence of the lone star tick (Amblyomma americanum) also had a high prevalence of Theileria sp. infections in the deer (Kuttler et al., 1967). Adult lone star ticks that were infected as nymphs on Theileria cervi infected deer, transmitted the organism with a prepatent period of 14-21 days.

Theileria sp. was first described in deer in Oklahoma in 1969 (Barker et al., 1973) in captive deer that were part of a white-tailed deer\lone star tick study. The Theileria sp. was apparently transmitted from field collected ticks to non-infected fawns. Piroplasms appeared in the blood films of all tick-infested fawns and were not observed in the unexposed controls.

Extensive mortality of white-tailed fawns at Cookson Wildlife Refuge in eastern Oklahoma was reported to be due to heavy tick infestations (Hair, 1968; Bolte et al., 1970).

Barker et al. (1973), while studying hematological changes in tick infected fawns, stated that T. cervi appears to be a contributing factor to reduced hemoglobin and packed cell volume values in tick infested fawns.

The biology of the lone star tick has been studied extensively in eastern Oklahoma (Patrick et al., 1977; Semtner et al., 1971; Clymer, et al., 1970) The association

of the lone star tick and T. cervi with white-tailed deer has been well established. Theileria cervi piroplasm parasitemias as high as 27% have been demonstrated in captive, parasite free white-tail fawns infested with field collected adult lone star ticks from the Cookson Hills Game Refuge (Barker et al. 1973). Although piroplasm parasitemias of adult deer from this refuge are low (1% or less), the percentage of infected adult deer harvested from this area may be as high as 100% (Kocan, personal comm.). However, the relationship between T. cervi and the various stages of the lone star tick, the degree of infection in ticks and the prevalence of infected ticks has not been established. The high prevalence of T. cervi in white-tailed deer and large populations of both deer and ticks, makes the potential for high infection rates in the tick population is optimal.

Buscher and Tangus (1986), however, while working with Theileria parva, the causative agent for East Coast Fever in east and central Africa, found no correlation between the parasitemia of the bovid host and the degree of infection in adult ticks developing from nymphs imbibing this blood. These results are in contrast to findings by Purnell et al. (1974), who demonstrated that a parasitemia in calves above 40% led to higher infection in adult ticks. The relationship between the parasitemia of T. cervi in white-tailed deer in Oklahoma and the prevalence of

infection in ticks feeding on these deer has not been established.

The objectives of this study were divided into two categories:

I.) Field

a. Determine prevalence, intensity and abundance of infection in field collected ticks (nymphs and adults) from eastern Oklahoma from March to September, 1986.

b. Determine if there is a seasonal variation in infection of ticks.

c. Determine in what tick stage(s) the parasite overwinters.

II.) Laboratory

a. Determine if a minimum T. cervi piroplasm parasitemia in the deer is necessary for infection of the ticks.

b. Determine if the T. cervi piroplasm parasitemia in the host influences prevalence, intensity or abundance of infection in the tick.

c. Determine if the sex of the tick influences the transmission of T. cervi.

CHAPTER II

MATERIALS AND METHODS

Description of Facilities

Deer Facilities

A 6 hectare deer research facility was available for these studies. The facility consists of two, four-acre fenced pens, a barn with six 2x7 m enclosed runs and four cement floored holding pens (7x7 m ea.).

Tick Facilities

The Oklahoma State University Department of Entomology Tick Laboratory is 1225 sq. meters and provides facilities for rearing and holding ticks. The laboratory has facilities for feeding ticks on rabbits or sheep to obtain desired developemental stages. Humidity chambers were available for holding ticks while molting (Patrick, et al.,1975).

Field Collection of Ticks and Tick Processing

Amblyomma americanum were collected from the field once

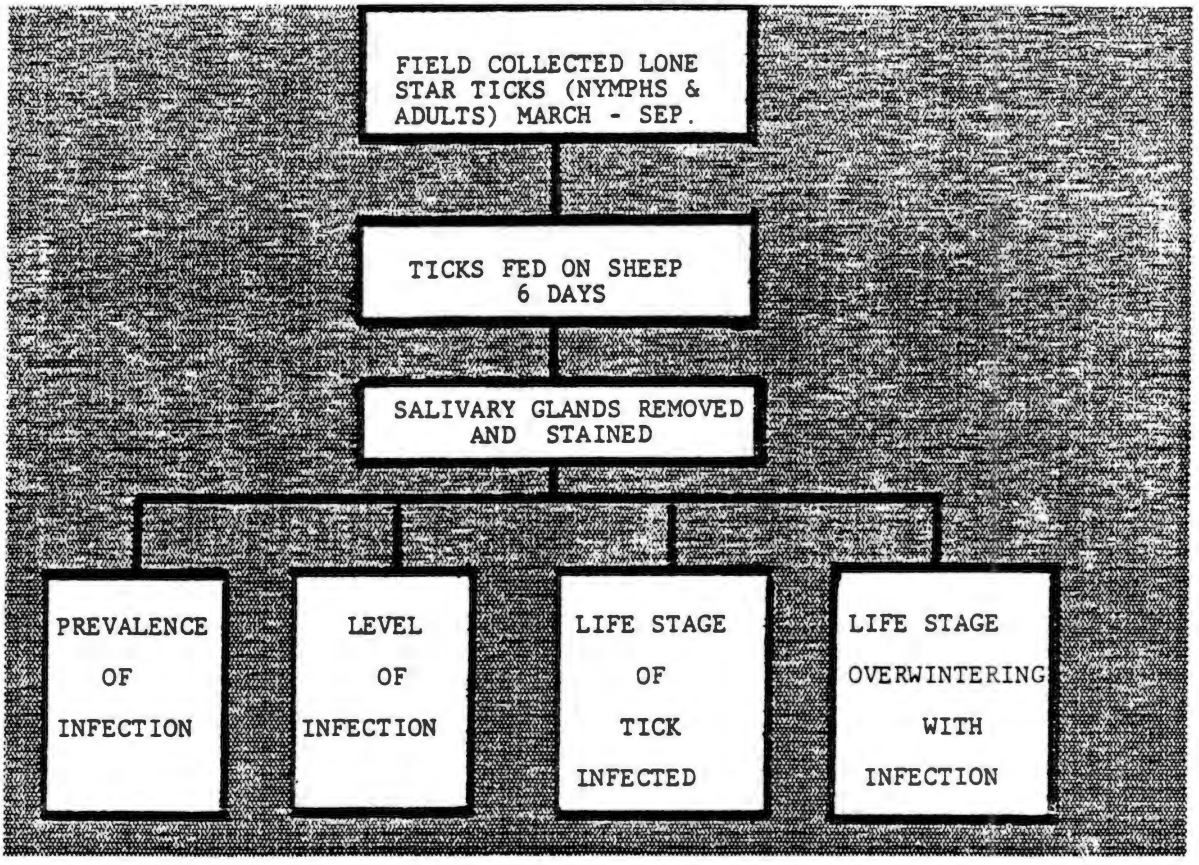
a week from March to September 1986. Ticks were collected from Cookson Hills Wildlife Refuge, located in southeastern Cherokee County and southwestern Adair County in eastern Oklahoma.

Unfed nymph and adult lone star ticks, were collected using the CO_2 baited trap method described by Wilson et al. (1972). The ticks were collected from the ecotone (prairie-woods interface) areas because both ticks (Semtner et al. 1971) and deer (Bartlett, 1938) are abundant in this habitat. The ticks were transported to the laboratory and placed in a stockinette cell attached to a closely shorn sheep; those that did not attach were removed after 24 hours. The ticks were allowed to feed for six days at which time all ticks were removed. Using a dissecting microscope, the dorsal and ventral halves of the ticks exoskeleton were separated with a razor blade and the salivary glands were dissected with a pair of forceps and spread on a microscope slide in order to attain a single acinar layer. The glands were air dried and stained with methyl green pyronin as described by Walker et al. (1979). The salivary glands were examined with light microscopy, using 10x objective, for the presence of the infected acini.

Quantitative Studies of Infection
in Field Collected Ticks.

Observations of salivary glands from field collected

Figure 1. Experimental design for collection, preparation and examination of field collected *Amblyomma americanum* from Cookson Hills Wildlife Refuge, Oklahoma, 1986.


FIELD COLLECTED LONE STAR TICKS (NYMPHS & ADULTS) MARCH - SEP.
TICKS FED ON SHEEP 6 DAYS
SALIVARY GLANDS REMOVED AND STAINED
PREVALENCE OF INFECTION
LEVEL OF INFECTION
LIFE STAGE OF TICK INFECTED
LIFE STAGE OVERWINTERING WITH INFECTION

ticks were used to determine: 1.) prevalence of infection; 2.) tick stages in which the parasite overwintered; and 3.) stage in tick life cycle in which the highest prevalence, intensity and abundance of infection were observed. A Z-test was used to compare prevalence of infection from each month of collection. Values of 1.96 or greater at 5% were considered significant.

Conformation of Infectivity of
Field Collected Ticks.
Transmission of T. cervi.

To determine if the infected acini that have been observed in field collected ticks were T. cervi, a stabilate made from sporozoites in salivary glands from field collected A. americanum (Kocan, et al. 1987) was injected subcutaneosly into a susceptible white-tailed deer previously determined to be uninfected by repeated blood film examination.

Experimental Infection of Ticks.

Four white-tailed deer fawns were used in the study. Two fawns were naturally infected from the field, and two were splenectomized and experimentally infected. The fawns were identified according to their piroplasm parasitemia, namely, 1%, 2%, 6% or greater than 20%. Each of the fawns was placed in a solid wooden box (0.8x1.2x1.0 m) for 24 hours with approximately 1000 lone star nymphs. The

infested fawn was then placed in a heavy wire cage (1.0x0.7x0.9 m) over a stainless steel pan. The pan was lined with newspaper and adhesive tape to catch the replete nymphs. The nymphs were collected 1 to 2 times per day, counted, placed in paper cartons and stored in a humidity chamber (90-98% relative humidity, 25 C with a 14 hour light-dark photophase).

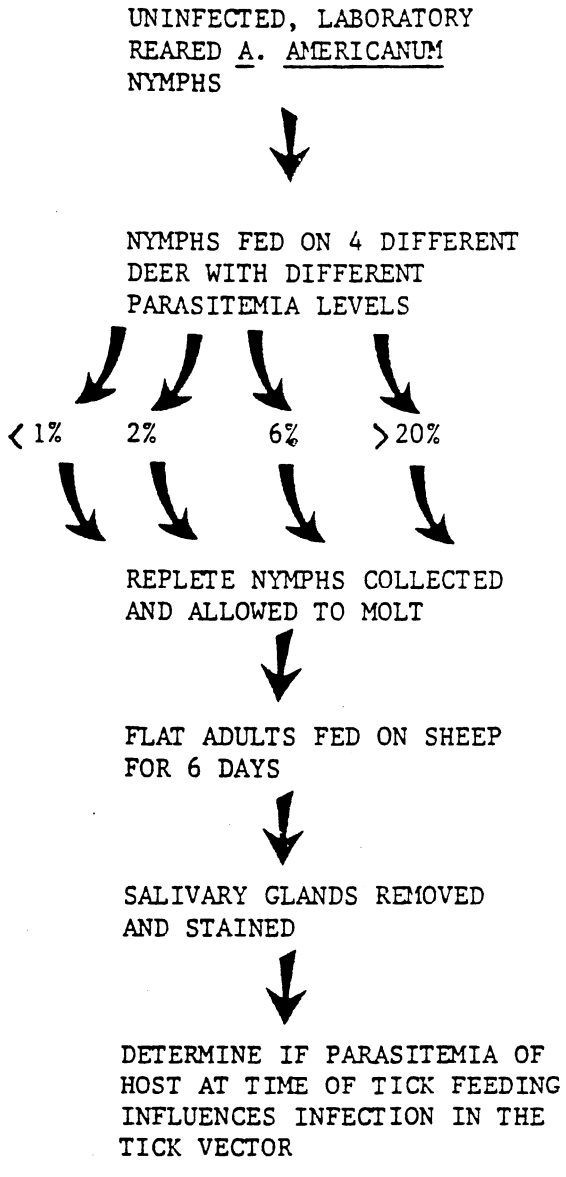
Each fawn remained in the cage for 7 days or until all of the nymphs became replete. The fawn was then dusted with an acaricide powder, bled to determine post-repletion parasitemia and returned to the deer research facility.

Quantification of Experimental Infection of Ticks.

After molting, adult lone star ticks were placed in a stockinette on a closely shorn sheep and allowed to feed for six days. After ticks were removed, the salivary glands were dissected and stained as described above. Prevalence (no. of infected ticks\no. of ticks examined), intensity (no. of infected acini\no.infected ticks), and abundance (no. of infected acini\no. of ticks examined) were determined.

An analysis of variance test was used to compare prevalence, intensity and abundance at different levels of piroplasm parasitemias. P values less than P=0.05 were considered significant.

Figure 2. Experimental design for infection of laboratory reared Amblyomma americanum nymphs by feeding nymphs on four deer with different parasitemia levels.


UNINFECTED, LABORATORY REARED A. AMERICANUM NYMPHS
NYMPHS FED ON 4 DIFFERENT DEER WITH DIFFERENT PARASITEMIA LEVELS
< 1%
2%
6%
> 20%
REPLETE NYMPHS COLLECTED AND ALLOWED TO MOLT
FLAT ADULTS FED ON SHEEP FOR 6 DAYS
SALIVARY GLANDS REMOVED AND STAINED
DETERMINE IF PARASITEMIA OF HOST AT TIME OF TICK FEEDING INFLUENCES INFECTION IN THE TICK VECTOR

CHAPTER III

RESULTS

Field Collected Ticks

Of the 150 ticks that were collected from Cookson Hills Wildlife Refuge in eastern Oklahoma the prevalence of infection with Theileria cervi for all ticks examined was 11.3% (17/150). The overall intensity of infection was 8.3 infected acini/ infected tick and the abundance of infection was 0.95 infected acini/tick examined (Table 1).

Female lone star ticks had the highest prevalence, intensity and abundance of infection. Of 87 females collected, 16.1% (14/87) were infected with T. cervi with a mean of 10 infected acini/infected female, and an abundance of infection of 1.6 infected acini/female examined. The greatest intensity observed in the field collected females was 48 infected acini\tick.

Nymphal ticks had the next highest prevalence of infection of the field collected ticks. Of 37 nymphs examined, 8.1% (3/37) were infected with a mean intensity of infection of 1.0 infected acini/infected nymph and an abundance of 0.08 infected acini/nymph examined. No infected male ticks were found in the 26 examined.

TABLE I

THEILERIA CERVI INFECTIONS IN FIELD COLLECTED *AMBLYOMMA AMERICANUM* FROM COOKSON HILLS WILDLIFE REFUGE, OKLAHOMA, 1986

sex/stage	sample size	prevalence	intensity	abundance
female	87	16.1	10	1.6
male	26	0.0	0	0.0
nymph	37	8.1	1	0.08
total	150	11.3	8.3	0.95

An increasing percent of infected females per month was observed as tick activity increased (Fig. 3). The first infected ticks observed were unfed females collected the second week of March. Of all ticks collected in March, 9.0% (3/33) were infected. In April, 11.3% (6/53) were infected. During May, the prevalence of T. cervi infection was 18.5% (5/27). After May, the prevalence of infection in female ticks decreased. In June, 5.8% (1/17) of the ticks examined were infected, and no infected females were found in July. By the beginning of August, adult tick activity had decreased to the point were no more adults were collected. During the spring-summer collection period, a total of 3 infected nymphs were found, one in April and two in August. Statistical analysis of monthly prevalence values revealed however, that the monthly increases observed in prevalence were not statistically significant.

Experimentally Infected Ticks

When ticks were allowed to feed on deer with varying parasitemias, only ticks feeding on deer with a parasitemia of greater than 1% became infected (Figure 4). In the groups in which ticks fed on deer with parasitemias greater than 1%, both males and females became infected, with the highest prevalence (100%) of infection being observed in females which fed on deer with a 6% piroplasm parasitemia.

Among infected ticks, both males and females had high but varying degrees of infection (Fig. 5.). Statistical

Figure 3. Seasonal variation in prevalence of infection of female Amblyomma americanum with Theileria cervi from Cookson Hills Wildlife Refuge, Oklahoma, 1986.

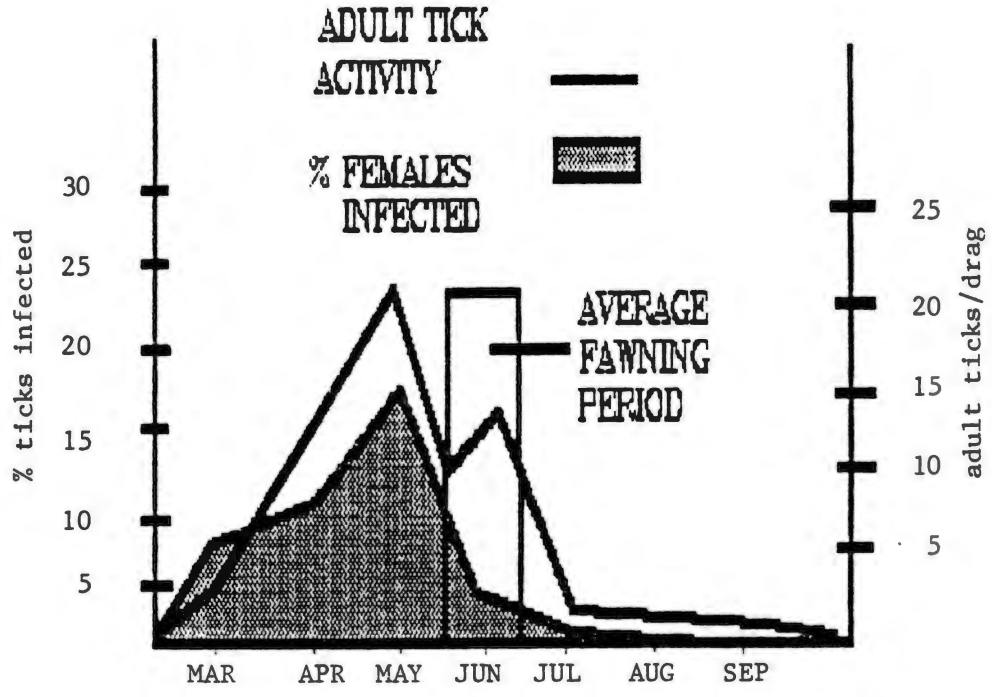
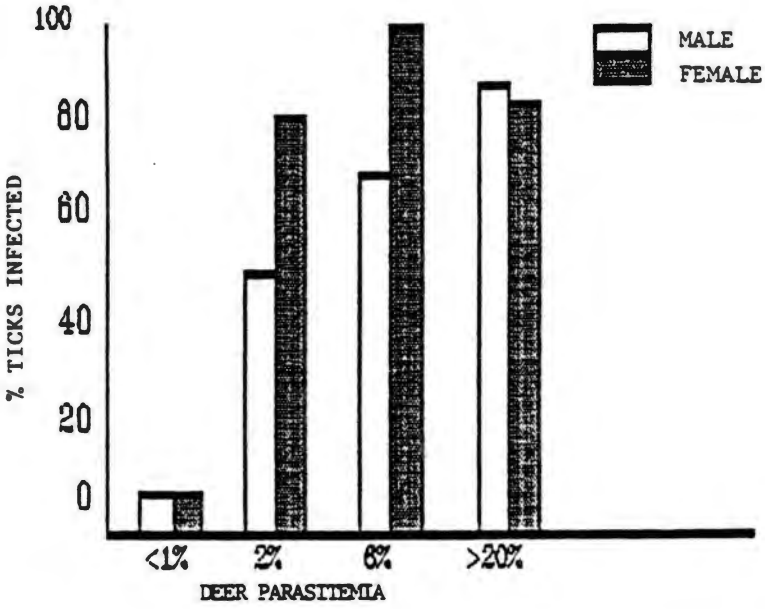
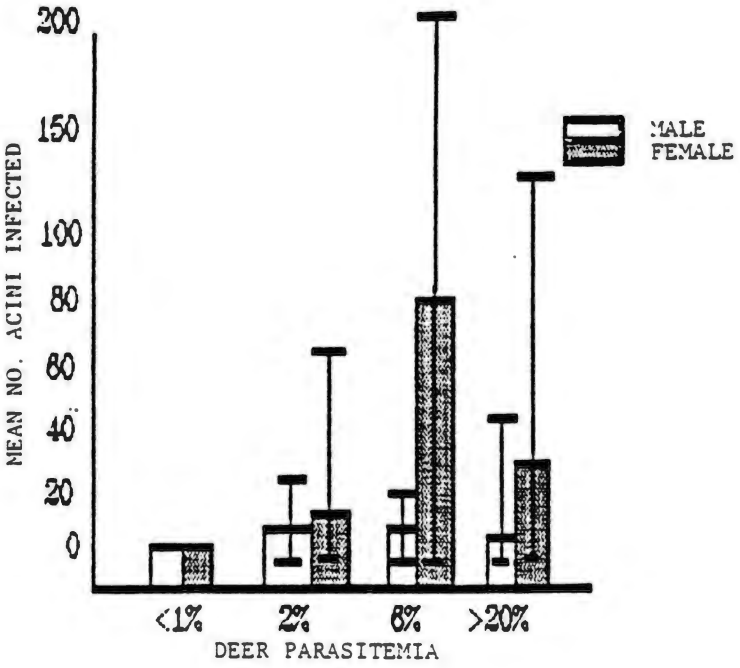

ADULT TICK ACTIVITY
% FEMALES INFECTED
AVERAGE FAWNING PERIOD
% ticks infected
adult ticks/drag
30
25
20
15
10
5
25
20
15
10
5
MAR
APR
MAY
JUN
JUL
AUG
SEP

Figure 4. Prevalence of infection of adult Amblyomma americanum after exposure to deer with varying parasitemias of Theileria cervi.

Figure 5. Mean intensity of infection of adult Amblyomma americanum after exposure to deer with varying parasitemias of Theileria cervi. Vertical lines represent variation of intensity of infection within each group.


100
80
60
40
20
0
% TICKS INFECTED
MALE
FEMALE
<1%
2%
6%
>20%
DEER PARASITEMIA


200
150
100
80
60
40
20
0
MEAN NO. ACINI INFECTED
MALE
FEMALE
<1%
2%
6%
>20%
DEER PARASITEMIA

evaluation of the intensity of infection comparing males and females showed that females acquired statistically heavier infections than did males (P=.005). Females feeding on the fawn with the 6% parasitemia level had infections as high as 212 infected acini/tick. Ticks which fed on the fawn with a greater than 20% parasitemia acquired a high intensity of infection (Fig. 5) but the intensity of infection was not significantly different from that seen in the ticks which fed on the deer with a 2% parasitemia. The greatest intensity of infection was seen in females which fed on the deer with a 6% parasitemia. In this group, the intensity of infection was greater than any other group (P=.041). The mean intensity of infection observed for male and female ticks from this group was 12.3 and 82.7 infected acini respectively. This group also contained the greatest variation in female tick infectivity, ranging from 1 infected acinus/tick to 212 infected acini/tick. This was the only group in which all experimentally exposed ticks examined became infected.

CHAPTER IV

Discussion

Evaluation of data from field collected and experimentally infected Amblyomma americanum suggests that the epidemiology of Theileria cervi is coordinated with the seasonal activity pattern of the lone star tick and suggests that the propagation of this parasite is dependent on environmental conditions and infection levels in the definitive host. Results indicate that the female tick is the primary vector of this parasite in the deer population. The prevalence of the parasite in the wild tick population closely follows the adult activity pattern at Cookson Hills Refuge (Fig. 1.) for a spring-summer activity season (Hair and Bowman, 1986). As the level of adult tick activity increases, the number of ticks with infected acini increases.

In the present study, collection of field ticks began in early March and ended in late August with the first infected tick observed in mid-March. Based on lone star tick feeding behavior and activity patterns (Patrick et al. 1977) this tick was either infected as a nymph while feeding the previous spring or fall which then molted to an adult and remained in diapause until the spring of the following

year, or was a "second year" active female that became infected two summers previously. This finding supports and extends those of Durham et al. (1976) and Barker et al. (1973) that T. cervi can overwinter in females. Additionally, since females were the common infected life stage recovered from the field (Table 1), it appears that overwintering females, which were infected as nymphs, play the major role in transmitting the parasite to the definitive host. Although infected field collected nymphs were observed it is not known at this time if nymphs, due to their different feeding behavior and short engorgement time, are able to transmit mature sporozoites to the deer host.

Results of experimental laboratory infection of ticks suggest that a minimum piroplasm parasitemia in the deer is necessary for infection of ticks with T. cervi (Figures 4 and 5). By comparison, Buscher et al.(1986), working with the transmission of Theileria parva in Rhipicephalus appendiculatus, found no correlation between the level of parasitemia of the host and the degree of infection in adult ticks imbibing this blood. Purnell et al.(1974), also working with T. parva, did report a difference but only in ticks which fed on calves with parasitemias of greater than 40%.

In the present study, it was found that the parasitemia level can be a critical factor in acquisition of infection by the tick. In ticks allowed to feed on deer with varying parasitemias, only those that fed on deer with a parasitemia

greater than 1% became infected. This suggests that a critical parasitemia at or near 1% in the host is necessary for the tick to acquire the infection.

Ticks which fed on deer with parasitemias above 1% were likely to become infected (Figure 4). Statistical analysis indicates that the "optimum" parasitemia in the deer is 6% for the greatest intensity of infection in the ticks.

Although no field collected male ticks were found infected, laboratory studies showed that males are capable of acquiring heavy infections. The absence of infected males among the field collected ticks may be related to the small sample of males examined due to high mortality rates after trapping or the intermittent feeding behavior of lone star males (Patrick and Hair, 1977). Since laboratory studies showed that males are able to acquire infections, perhaps there are other biological reasons why no infected males were recovered. The inability of the parasite to survive for long periods of time in a male tick is a possibility or perhaps the males had already fed one or more times and lost the infection.

Results from the present study can be projected to help explain the epidemiology of transmission of this parasite in nature. Previous studies on Cookson Hills Refuge indicate that adult deer normally harbor parasitemias of 1% or less (Barker et al.,1973). The results of the present study show, however, that laboratoy reared lone star ticks feeding on deer with less than a 1% parasitemia do not become

infected (Figures 4 and 5). If the results of the present study can be projected to natural conditions, it appears that the role of the adult deer as a source of infection for ticks is minimal. However, these findings do not preclude the possibility that unknown fluctuations in parasitemia levels may occur in deer during the tick feeding season. Data available at present, however, indicates that 1-2 week old intact fawns can be experimentally infected with T. cervi by allowing adult lone star ticks, exposed as nymphs, to feed and that they can harbor parasitemias as high as 27% (Barker, et al., 1973).

Heavy tick burdens and mortality of white-tailed deer fawns are well documented on Cookson Hills Wildlife Refuge (Bolte, et al., 1970; Barker, et al., 1973). If one considers the behavior of neonatal fawns, the potential for acquiring heavy infections is evident. New born fawns spend most of the day hidden in underbrush and carry on a minimum amount of grooming. They move about only to feed or change location, particularly during the first week after birth (Jacobsen, 1979). They often hide in ecotone areas that provide the best cover and also harbor the largest number of ticks (Hair and Bowman, 1986). In essence, the timing of spring tick activity and fawning activity inhance the opportunity for heavy infestations of fawns with infected female lone star ticks. This in turn can result in high parasitemias in the fawns as soon as 11 days after tick

attachment. These fawns can then serve as ideal hosts for nymphs and the perpetuation of the T. cervi cycle.

CHAPTER V

Summary and Conclusions

Studies of the suseptibility of Amblyomma americanum to infection with Theileria cervi can lead to a better understanding of the epidemiology of this parasite. While T. cervi is not considered pathogenic in healthy adult white-tailed deer, its effect on neonatal fawns and malnurished adult deer is not clear.

A total of 150 lone star ticks were collected from Cookson Hills Wildlife Refuge in eastern Oklahoma in an effort to determine prevalence, intensity and abundance of infection in field collected lone star ticks.

In laboratory experiments, four groups of laboratory reared lone star nymphs were fed on four Theileria infected white-tailed deer with different parasitemias to determine if there is a critical parasitemia in the deer necessary to infect the ticks.

Prevalence of infection in all field collected ticks was 11.3%. All but three of these infected ticks were adult females. Three infected nymphs were collected; no infected males were found. An increasing percent of infected females per month was observed as tick activity increased. Prevalence of infection peaked in May with 5/27 female ticks

being infected. After May, prevalence of infection in female ticks decreased. However, monthly increases in prevalence of infection were not statistically significant.

Female field collected ticks showed the greatest prevalence, intensity and abundance of infection. Intensity of infection in field collected ticks ranged from one infected acini/tick to 48 infected acini/tick. Theileria cervi was found to overwinter in adult female ticks.

Experimental laboratory infected ticks became heavily infected. A minimum piroplasm parasitemia in the deer of greater than 1% was necessary for infection of the ticks with T. cervi.

The level of T. cervi piroplasm parasitemia in the deer host influenced prevalence, intensity and abundance of infection in the tick. Ticks which fed on a deer with a parasitemia of less than 1% showed no infection. Ticks which fed on deer with parasitemias of 2%, 6% and greater than 20% showed varying degrees of intensity of infection. This ranged from no infected to heavy infection in the individual ticks. Ticks which fed on the deer with a parasitemia of 6% showed the greatest prevalence, intensity and abundance of infection. The intensity of infection in this group of ticks was statistically greater than any other group ($P=.041$). Additionally, females acquired statistically heavier infections than did males ($P=.005$).

Data suggests that, due to their low parasitemias, adult white-tailed deer on the refuge may not be the primary

source of infection for the ticks. Fawns, which have been shown under experimental conditions to develope high parasitemias, could act as the primary source of infection for lone star ticks.

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VITA

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